The *p*-Hydroxybenzyl Alcohol-Increased Passive Avoidance Response in Rats: Interactions with Serotonergic and Adrenergic Receptors

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ABSTRACT

To assess the role of the serotonergic system in the *p*-hydroxybenzyl alcohol (HBA)-increased passive avoidance response in rats, we examined the effect of HBA on *p*-chloroamphetamine (PCA)-induced impairment of avoidance by challenging with the selective 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide (8-OH-DPAT) and the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) as well as with selective 5-HT_1 (pindolol) and 5-HT_2 (ritanserin) antagonists. It was observed that pretraining administration of HBA at doses of 1-25 mg/kg significantly counteracted the PCA-induced shortening of the step-through latency in the retention test. Pretraining administration of piracetam at 100 mg/kg also improved the PCA-induced shortening of the step-through latency. The counteracting effect of HBA was observed to be significantly antagonized by the 5-HT_{1A}-selective agonist 8-OH-DPAT, but potentiated by the 5-HT_1 antagonist (±)-pindolol. The effect of HBA was also significantly antagonized by the 5-HT_2-selective agonist DOI, but potentiated by the selective 5-HT_2 antagonist, ritanserin. The counteracting effect of HBA was also potentiated by the α_1-blocker prazosin, but not by the α_2-blocker yohimbine and the β-blocker propranolol. The present results suggest that the beneficial effect of HBA on the PCA-induced impairment of avoidance may be related to decreased serotonergic activity by 5-HT_{1A} and 5-HT_2 receptors, and also to decreased adrenergic activity by α_1 receptors.

Key words: *p*-Hydroxybenzyl alcohol; *p*-Chloroamphetamine; Passive avoidance task; Serotonergic systems; Adrenergic systems.

INTRODUCTION

Gastrodia elata Blume (Orchidaceae) (GE) is used to cure neurasthenia, neurasthenic syndrome, and vasoneuronal headaches in traditional Chinese medicine.[1] Kao *et al.* have shown that aqueous extracts of GE could improve the D-galactose-induced impairment in mice[2] and the deficiency of the step-down passive avoidance performance in senescent mice.[3] Phytochemical researchers have found that gastrodin and its aglycone (*p*-hydroxybenzyl alcohol) are major constituents of GE rhizoma.[4] In our previous studies, subacute administration of the methanol extract of GE and its constituents - gastrodin and *p*-hydroxybenzyl alcohol, which were separated from *n*-butanol and ethyl acetate fractions of methanol extracts, were observed to counteract the scopolamine-induced deficit in passive avoidance performance in rats.[5] Furthermore, pretraining administration of *p*-hydroxybenzyl alcohol prevented cycloheximide-induced impairment of memory consolidation by acting through 5-HT_{1A} and 5-HT_2 serotonergic receptors (unpublished results).

Learning acquisition represents the experience-dependent modification of enduring internal representations. It involves the activation, by neurotransmitters such as acetylcholine, dopamine and serotonin, of receptor-linked enzymes responsible for the synthesis of intercellular messangers.[6-7] Much evidence suggests that 5-HT neurons participate in learning and memory in humans and animals.[8-9] Recent studies show a consistent trend in which the central 5-HT system plays an inhibitory role in avoidance and aversive conditioning tasks.[10] In fact, 5-HT antagonists facilitate learning acquisition,[11] while the 5-HT precursor 5-hydroxytryptophan and the serotonin releaser *p*-chloroamphetamine, when administered before training, increase 5-HT transmission and impair learning acquisition in rodents.[12-13] Furthermore, two main types of 5-HT receptor have been identified, and termed 5-HT_1 and 5-HT_2 receptors.[14] 5-HT_1 and 5-HT_2 receptors also play an inhibitory role in learning and memory. Recent studies have found that 5-HT_1 agonists impair the performance in various experimental tasks such as passive avoidance,[15] radial arm maze[16] and water maze[17], and 5-HT_2 agonists impair the performance in the passive avoidance task.[15]

In the present study, we attempted to investigate whether HBA attenuates PCA-induced impairment of learning acquisition and evaluate the effective use of piracetam as a positive control. To demonstrate the action mechanism of HBA, we further examined combination of HBA with the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide and antagonist pindolol as well as with the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane and antagonist ritanserin on the counteracting effect of HBA on the PCA-induced impairment of the passive avoidance response.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing 200-250 g, were housed in groups of six with free access to food and water in a regulated environment (23 ± 1 °C) with a 12 h light-dark cycle (8:00 to 20:00, light). Each experimental group included 12 to 18 rats.

Chemicals

p-Hydroxybenzyl alcohol (HBA), piracetam (PIR), *p*-chloroamphetamine hydrochloride (PCA), propranolol (PROP) and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) were dissolved in 0.9 % saline. 8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT) was dissolved in 0.9 % saline containing 0.5 % ascorbic acid. (±)-Pindolol (PIN) was dissolved with a small amount of 0.03 N hydrochloric acid in 0.9 % saline. Ritanserin (RIN) was dissolved in distilled water along with a small amount of lactic acid, and sodium bicarbonate was used to adjust the pH of the solution to about 4. Prazosin (PRA) and yohimbine (YOH) were dissolved in 0.5 % carboxymethylcellulose. DOI and ritanserin were purchased from Research Biochemicals International (Natick, MA, USA). All other drugs were purchased from Sigma Chemical Co. (Louis, MO, USA).

Apparatus

Memory processes including acquisition, consolidation and retrieval were assessed with a step-through passive avoidance task. Rats were trained in a step-through passive avoidance task. The apparatus consisted of two compartments having a steel-rod grid floor (36 parallel steel rods, 0.3 cm in diameter set 1.5 cm apart). One of the compartments (48 × 20 × 30 cm) was equipped with a 20 W lamp located centrally at a height of 30 cm, and the other compartment of same size was dark, connected through a guillotine door (5 × 5 cm). The dark room was used during the experimental sessions that were conducted between 09:00 and 17:00 hours.

Administration of a Brief Shock to Rats

During the trial training, the guillotine door connecting the light and dark compartments was kept closed. After each rat was placed in the light compartment with its back to the guillotine door, the door was opened and the time (step-through latency, STL) taken by the rat to enter the dark compartment was measured with a stopwatch. Once the rat entered the dark compartment, the door was closed. An inescapable scrambled footshock (1 mA for 2 s) was then delivered through the grid floor. The rat was removed from the dark compartment 5 s after administering the shock. The rat was then put back into its home cage until

the retention trial, which was carried out twenty-four hours later. The rat was once again placed in the light compartment and as in the case of the training trial, the guillotine door was opened and the step-through latency was recorded and used as a measure of retention.[18] An upper cut-offtime of 300 sec was set.

HBA and Piracetam on PCA-Induced Impairment

In the first experiment, PCA (2.5 mg/kg, i.p.) was administered 30 minutes before the training trial.[13] HBA (0.5, 1, 5 and 25 mg/kg, p.o.) was administered to PCA-treated rats 1h before the training trial. Piracetam (50, 100 and 300 mg/kg, p.o.) was also administered to PCA-treated rats 1h before the training trial.

5-HT Agonists and Antagonists on the Counteracting Effect of HBA on PCA-Induced Impairment

HBA (1 mg/kg, p.o.) was administered to PCA-treated rats 1h before the training trial. 8-OH-DPAT (0 025 mg/kg, i.p.),[15] PIN (10 mg/kg, i.p.),[19] DOI (0.02 mg/kg, i.p.)[15] and RIN (0.25 mg/kg, i.p.)[20] were administered 30 minutes before the training trial.

Adrenergic Blockers on the Counteracting Effect of HBA on PCA-Induced Impairment

HBA (1 mg/kg, p.o.) was administered to PCA-treated rats 1h before the training trial. PRA (0.5 mg/kg, i.p.),[19] YOH (0.3 mg/kg, i.p.)[21] and PROP (3 mg/kg, i.p.)[22] were administered 30 minutes before the training trial.

Effect of HBA and Other Drugs on Step-Through Latencies of Unshocked Rats

To evaluate the effect of various drug combinations on motor activity in the passive avoidance task, the same experimental steps were followed as described in the above section with the exception that rats were not subjected to a 1.0 mA inescapable footshock during the training period. Twenty-four hours later, the retention trial was carried out and the step-through latency was recorded.[23] The rats were given 2.5 mg/kg PCA in combination with the above mentioned drugs.

Pain Sensitivity

The thresholds for flinching, jumping or vocalization produced by electric shock were measured by using the passive avoidance task. Each rat was placed in the dark compartment of the passive avoidance task apparatus and the shock intensity was manually raised stepwise from 0.5 to 1.0 mA in increments of 0.1 mA until either a flinch, jump or vocali-

zation was observed. The duration of the shock was 2 s and the intershock interval was 15 s. The point at which the rat exhibited vocalization was recorded.[24] The rats were orally given doses of 0.5, 1, 5 or 25 mg/kg HBA 1h before the training trial.

Statistical Analysis

All data obtained during the passive avoidance task was expressed in terms of medians and interquartile ranges and further analyzed by using a Kruskal-Wallis non-parametric one-way analysis of variance, followed by Mann-Whitney's U-test. In addition, the data collected during motor activity and shock sensitivity was analyzed using a one-way analysis of variance, followed by Duncan's multiple range test. The criterion for statistical significance was $P < 0.05$ in all the above statistical evaluations.

RESULTS

Effects of HBA and Piracetam on PCA-Induced Avoidance Impairment

It was observed that administration of PCA (2.5 mg/kg, i.p.) before training remarkably shortened the step-through latency period. As shown in Fig. 1, PCA-induced shortening of the step-through latency period was reversed by administration of 1-25 mg/kg HBA before training. On the other hand, administration of 100 mg/kg piracetam was required to significantly counteract the PCA-induced shortening of the step-through latency period (Fig. 1).

Effects of 5-HT Agonists and Antagonists on the Counteracting Effect of HBA on the PCA-Induced Avoidance Impairment

The 5-HT_{1A} selective agonist 8-OH-DPAT (0.025 mg/kg), which did not shorten the step-through latency period in the retention trial itself, attenuated the HBA inhibition of PCA-induced shortening of step-through latencies (Fig. 2 (A)). Pindolol (10 mg/kg), a 5-HT_1 antagonist, which did not have a significant anti-amnestic effect by itself, potentiated the HBA recovery of PCA-induced shortening of step-through latencies (Fig. 2 (B)). A 5-HT_2 selective agonist, DOI (0.02 mg/kg), which did not shorten the step-through latency, attenuated the HBA recovery from PCA-induced shortening of step-through latencies (Fig. 3 (A)). Ritanserin (0.25 mg/kg), a selective 5-HT_2 antagonist, which did not have a significant anti-amnestic effect on its own, potentiated HBA recovery from PCA-induced shortening of step-through latencies (Fig. 3 (B)).

Effects of Adrenergic Blockers on the Counteracting Effect of HBA on the PCA-Induced Avoidance Impairment

The α_1-blocker prazosin (0.5 mg/kg), which did not have a significant anti-amnestic effect on its own, potentiated the HBA inhibition of PCA-induced shortening of step-through latencies (Fig. 4). Yohimbine (0.3 mg/kg), a α_2-blocker, which did not have a significant anti-amnestic effect on its own, did not potentiate HBA recovery from PCA-induced shortening of step-through latencies (Fig. 5). Propranolol (3 mg/kg), a non-selective β-blocker, which did not have a significant anti-amnestic effect on its own, did not potentiate HBA recovery from PCA-induced shortening of step-through latencies (Fig. 6).

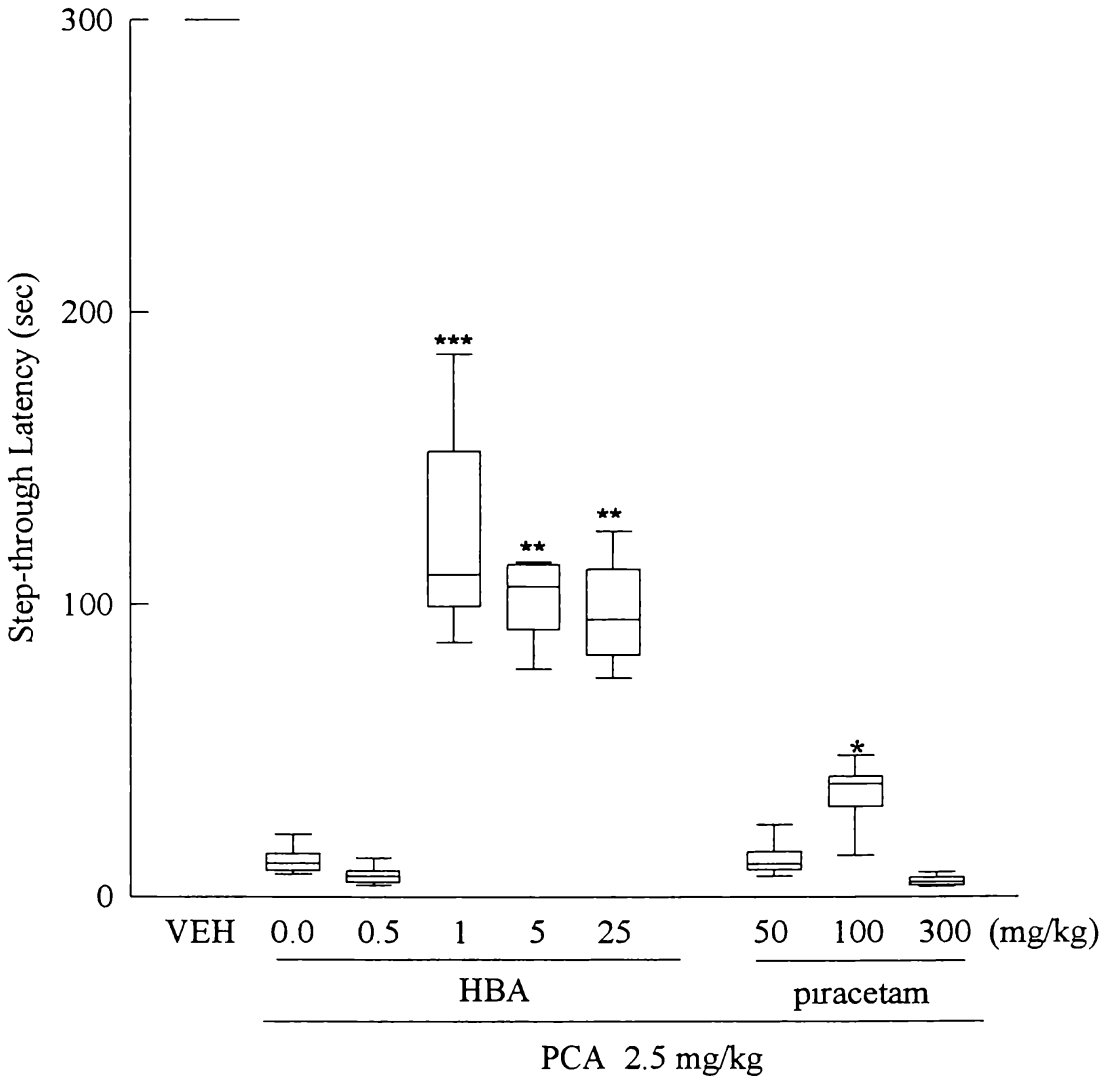

Fig. 1. Effects of *p*-hydroxybenzyl alcohol (HBA) and piracetam on PCA-induced impairment of passive avoidance response in rats. PCA was administered i.p. 30 minutes before the training trial. HBA and piracetam were administered p.o. 1 hr before the training trial. The VEH group was injected with 0.9 % normal saline. Each column shows the median and range of 12-18 rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the PCA group.

Effects of HBA and Other Drugs on Step-Through Latencies of Unshocked Rats

HBA had no effect on the step-through latencies of unshocked rats that were given PCA in the training and testing trial (Table 1). HBA in combination with other drugs also had no effect on the step-through latencies of unshocked rats that were given PCA (Table 2).

Effects of HBA and Other Drugs on Pain Sensitivity

As shown in Table 3, the flinch and vocalization thresholds of HBA at 0.5, 1.0, 5.0 and 25 mg/kg were not different from the thresholds of vehicle-treated rats. HBA in combina-

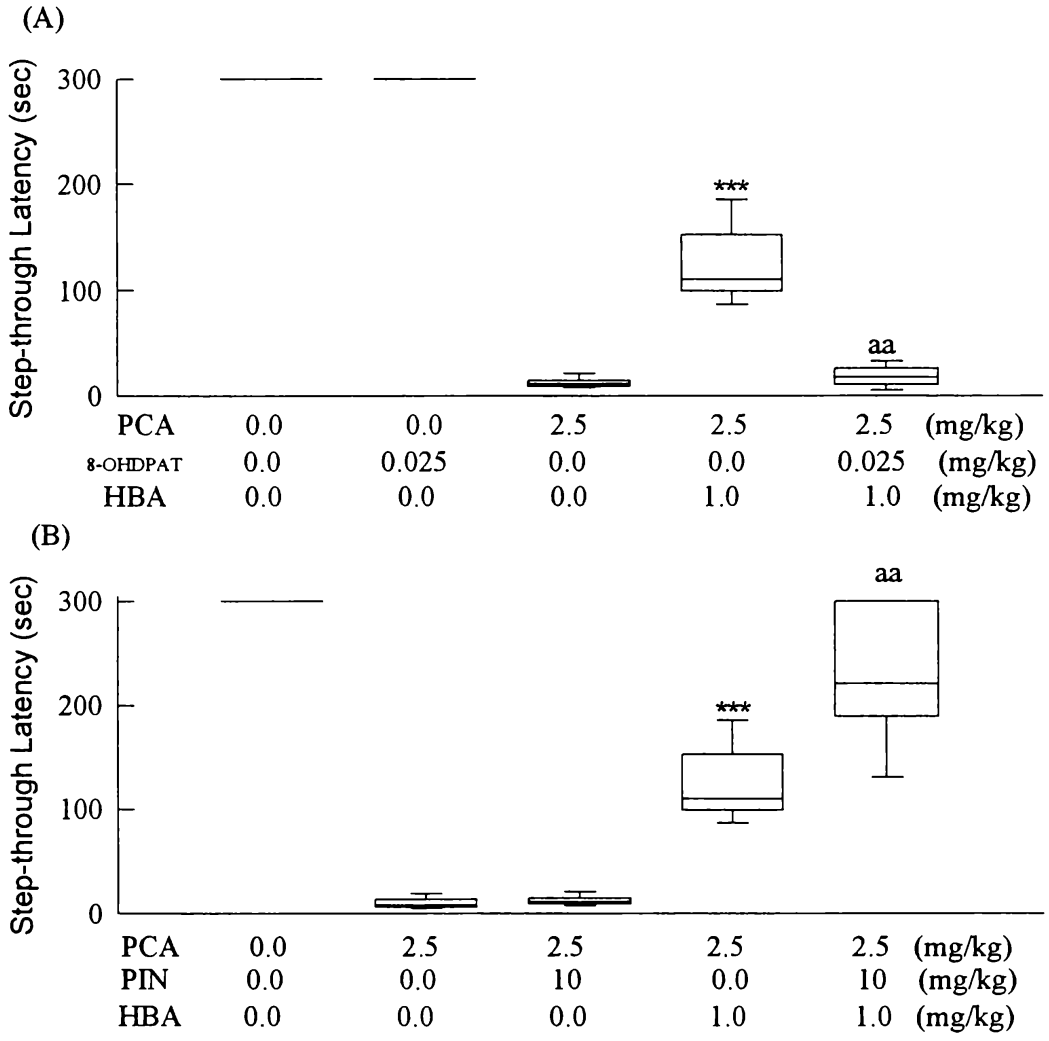

Fig. 2. Effects of (A) 8-OH-DPAT and (B) pindolol (PIN) on the *p*-hydroxybenzyl alcohol (HBA)-induced recovery from PCA-mediated impairment of the passive avoidance response in rats. 8-OH-DPAT, PIN and PCA were administered i.p. 30 minutes before the training trial. HBA was administered p.o. 1 hr before the training trial. Each column shows the median and range of 12-18 rats. *** $P < 0.001$ compared with the PCA group. aa $P < 0.01$ compared with PCA in combination with HBA.

tion with other drugs also had no effect on the flinch and vocalization thresholds of rats that were given PCA.

DISCUSSION

There is ample evidence indicating that the 5-HT neuronal system plays a significant role in learning and memory.[9] Stimulation of the 5-HT neuronal system is known to have a negative influence on learning and memory. The 5-HT releaser PCA in the present study also significantly shortened the step-through latency in the passive avoidance task of the retention trial. On the other hand, modification of the sensory, motivational or motor systems can in turn affect the acquisition of the avoidance response. Several aspects argue against

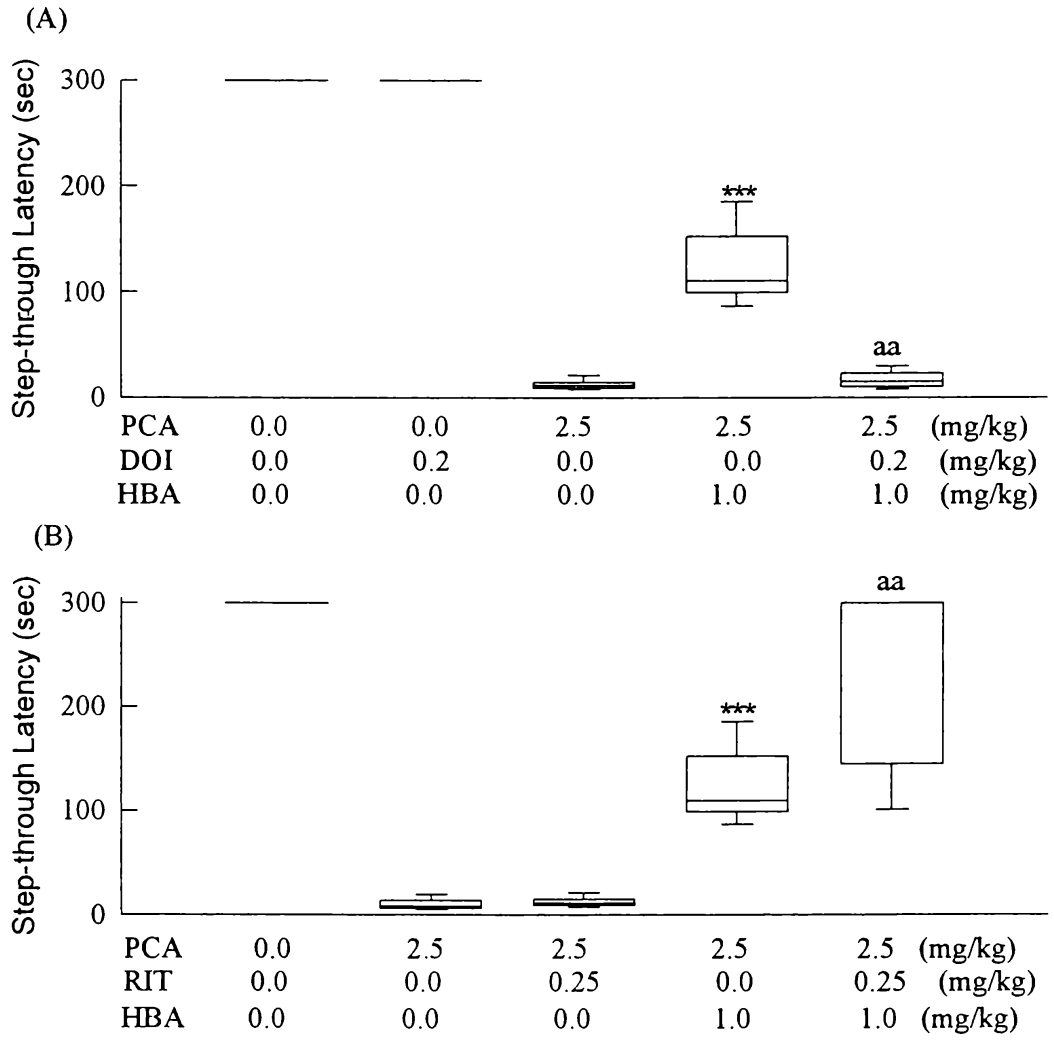

Fig. 3. Effect of (A) DOI and (B) ritanserin (RIT) on the *p*-hydroxybenzyl alcohol (HBA)-induced recovery from PCA-mediated impairment of the passive avoidance response in rats. DOI, RIT and PCA were administered i.p. 30 minutes before the training trial. HBA was administered p.o. 1 hr before the training trial. Each column shows the median and range of 12-18 rats. *** $P < 0.001$ compared with the PCA group. aa $P < 0.01$ compared with PCA in combination with HBA.

the possibility that the passive avoidance response observed in drug-treated animals is related to nociceptive stimuli or motor alteration during training. Although PCA can increase the threshold for pain sensitivity and motor activity,[13] these effects are distinct from the avoidance response deficit mediated by the descending 5-HT neurons. Our data indicate that PCA did not alter the nociception and motor activity. Our results therefore further support an inhibitory role for 5-HT in learning and memory. The nootropic piracetam counteracted the PCA-induced shortening of the step-through latency period and exhibited a well-known, bell-shaped dose-response curve similar to an early report of scopolamine-induced amnesia.[25] Its effect might mainly be related to an increased turnover rate of 5-HT.[26] HBA at doses of 1-25 mg/kg also counteracted the PCA-induced shortening of the step-through latency period in the passive avoidance task. Although some reports have pointed out that HBA has analgesic and sedative effects in mice,[27] the behavior and the vocalization thresh-

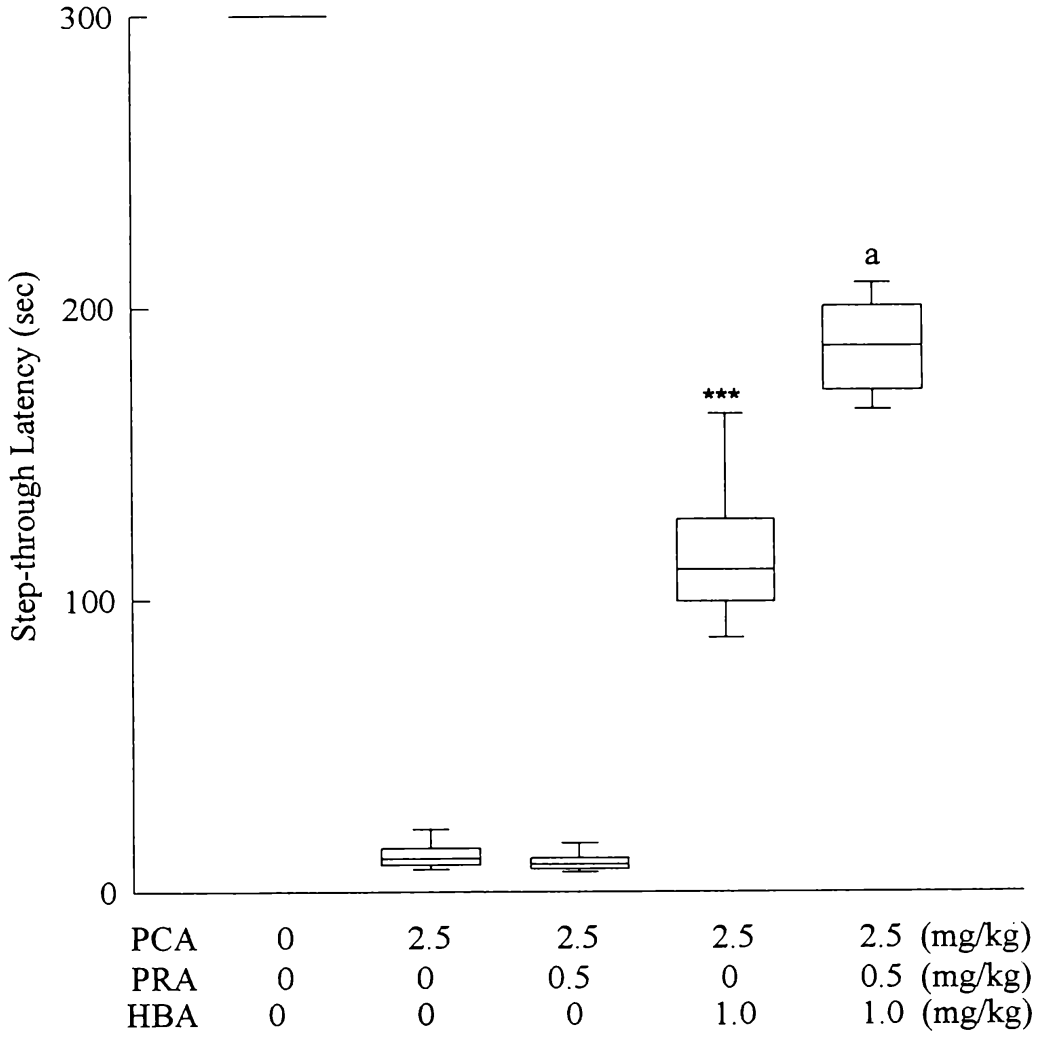

Fig. 4. Effect of prazosin (PRA) on the *p*-hydroxybenzyl alcohol (HBA)-induced recovery from PCA-mediated impairment of the passive avoidance response in rats. PRA and PCA were administered i.p. 30 minutes before the training trial. HBA was administered p.o. 1 hr before the training trial. Each column shows the median and range of 12-18 rats. ***P < 0.001 compared with the PCA group. a P < 0.05 compared with PCA in combination with HBA.

old of HBA-treated rats was normal in the training and retention trial. Therefore, our results demonstrate that the counteracting effect of HBA on the PCA-induced shortening of step-through latency is related to memory-related processes.

5-HT receptors can be divided into 5-HT_1 and 5-HT_2 receptors.[14] Recent studies have shown that both 5-HT_1 and 5-HT_2 receptors play a negative role in learning and memory.[15-17] The 5-HT_{1A}-selective agonist 8-OH-DPAT and 5-HT_2-selective agonist DOI impaired the passive avoidance response.[15] The counteracting effect of HBA on the PCA-induced shortening of step-through latencies was abolished by 8-OH-DPAT and DOI. Furthermore, the 5-HT_1-nonselective antagonist (±)-pindolol and the 5-HT_2 selective antagonist ritanserin have been shown to increase passive avoidance responses and ameliorate PCA-induced impairment of avoidance.[19-20] Doses of (±)-pindolol and ritanserin that did not increase passive avoidance behavior augmented the effect of HBA on the PCA-induced short-

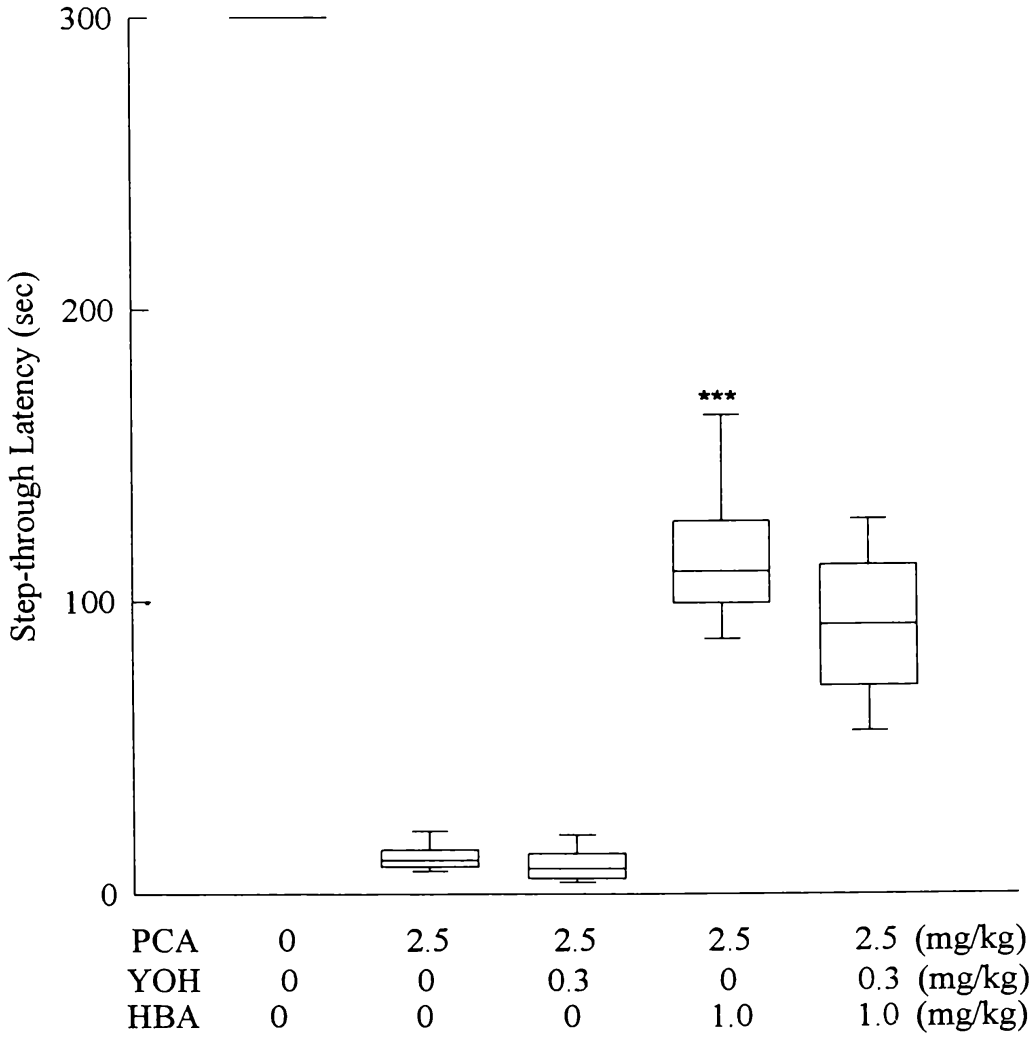

Fig. 5. Effect of yohimbine (YOH) on the *p*-hydroxybenzyl alcohol (HBA)-induced recovery from PCA-mediated impairment of the passive avoidance response in rats. YOH and PCA were administered i.p. 30 minutes before the training trial. HBA was administered p.o. 1 hr before the training trial. Each column shows the median and range of 12-18 rats. *** $P < 0.001$ compared with the PCA group.

ening of step-through latency period. Ögren (1985) and Matsuno *et al.* (1993) have demonstrated that PCA-induced impairment of the avoidance response was mainly mediated by increased 5-HT release by 5-HT_2 receptors.[13,20] However, 8-OH-DPAT can impair working memory and avoidance response by postsynaptic 5-HT_{1A} receptors.[15-17] In our studies, the ameliorating effects of HBA on the drug-induced impairment of the passive avoidance response could be amplified by 5-HT_{1A} and 5-HT_2 antagonists, but was reduced by 5-HT_{1A} and 5-HT_2 agonists. Thus, the ameliorating effect of HBA on the PCA-induced impairment of the passive avoidance response was similar to that of CXM, mediated mainly by blocking of 5-HT_{1A} and 5-HT_2 receptors. Recent studies have pointed out that other 5-HT receptor types such as 5-HT_3 and 5-HT_4 receptors also play an important role in learning and memory.[28,29] Therefore, the role of 5-HT_3 and 5-HT_4 receptors in the counteracting effect of

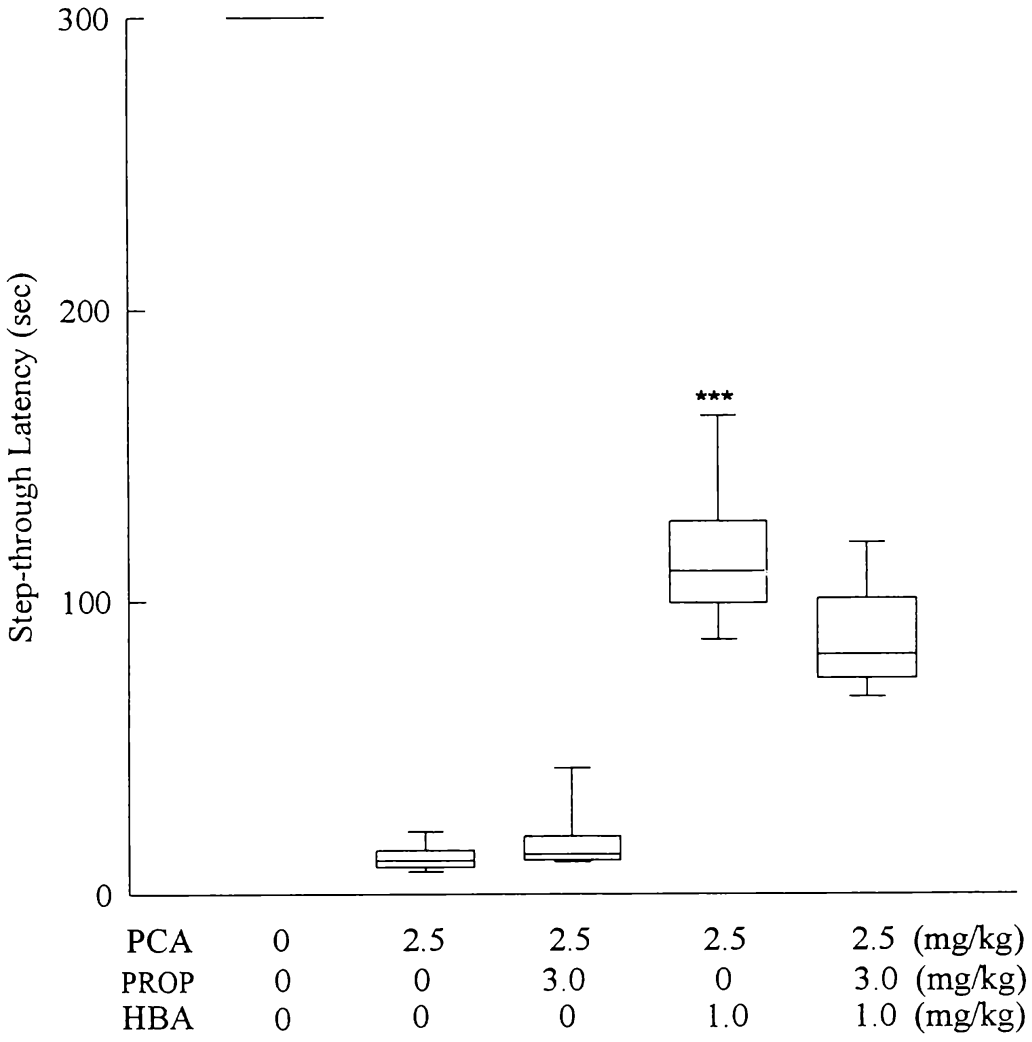

Fig. 6. Effect of propranolol (PROP) on the *p*-hydroxybenzyl alcohol (HBA)-induced recovery from PCA-mediated impairment of the passive avoidance response in rats. PROP and PCA were administered i.p. 30 minutes before the training trial. HBA was administered p.o. 1 hr before the training trial. Each column shows the median and range of 12-18 rats. *** $P < 0.001$ compared with the PCA group.

Table 1. Effects of *p*-Hydroxybenzyl Alcohol (HBA) on the Step-through Latency Period of Unshocked Rats Given PCA

Drug	Dose	Step-through Latency (sec)	
	(mg/kg)	Training trial	Testing trial
VEH		7.768 ± 1.427	8.903 ± 1.870
PCA	2.5	8.727 ± 3.216	9.748 ± 1.341
HBA + PCA	0.5	10.177 ± 2.682	12.545 ± 2.367
	1.0	10.290 ± 2.892	9.708 ± 3.050
	5.0	8.820 ± 2.108	6.553 ± 1.390
	25.0	11.173 ± 2.808	9.678 ± 1.291

PCA was administered i.p. 30 minutes before the training trial. HBA was administered p.o. 1 hr before the training trial. The results are expressed as mean ± S.E. of 8 rats per group.

Table 2. Effects of *p*-Hydroxybenzyl Alcohol (HBA) in Combination with Various Drugs on the Step-through Latency Period of Unshocked Rats Given PCA

Drug	Step-through Latency (sec)	
(mg/kg)	Training trial	Testing trial
Control	7.768 ± 1.427	8.903 ± 1.870
PCA 2.5	8.727 ± 3.216	9.748 ± 1.341
HBA 1.0 + PCA 2.5	10.290 ± 2.892	9.708 ± 3.050
HBA 1.0 + PCA 2.5 + 8-OHDPAT 0.025	12.506 ± 1.514	8.746 ± 2.143
PCA 2.5 + PIN 10	11.056 ± 2.576	9.108 ± 2.473
HBA 1.0 + PCA 2.5 + PIN 10	13.022 ± 2.341	9.491 ± 3.214
HBA 1.0 + PCA 2.5 + DOI 0.02	12.219 ± 3.205	8.814 ± 2.835
PCA 2.5 +RIN 0.25	10.736 ± 3.053	9.573 ± 1.974
HBA 1.0 + PCA 2.5 + RIN 0.25	10.134 ± 1.930	9.672 ± 3.519
PCA 2.5 + PRA 0.5	13.814 ± 3.730	8.971 ± 2.441
HBA 1.0 + PCA 2.5 + PRA 0.5	12.836 ± 2.917	10.017 ± 3.670
PCA 2.5 + YOH 0.3	13.509 ± 2.399	9.238 ± 2.874
HBA 1.0 + PCA 2.5 + YOH 0.3	16.171 ± 2.887	9.437 ± 2.691
PCA 2.5 + PROP 3	9.469 ± 2.805	8.882 ± 3.061
HBA 1.0 + PCA 2.5 + PROP 3	12.819 ± 3.442	9.024 ± 2.197

8-OH-DPAT, PIN, DOI, RIN, PRA, YOH, PROP and PCA were administered i.p. 30 minutes before the training trial. HBA was administered p.o. 1 hr before the training trial. The results are expressed as mean ± S.E. of 8 rats per group.

Table 3. Effects of *p*-Hydroxybenzyl Alcohol (HBA) on Electric Foot-shock in Rats

Drug	Dose (mg/kg)	Threshold (mA)	
		Flinch	Jump / Vocalization
Control		0.75 ± 0.03	0.88 ± 0.01
HBA	0.5	0.78 ± 0.02	0.86 ± 0.01
	1.0	0.74 ± 0.02	0.86 ± 0.01
	5.0	0.78 ± 0.02	0.88 ± 0.01
	25.0	0.76 ± 0.02	0.88 ± 0.02
HBA + PCA 2.5	0.5	0.79 ± 0.04	0.86 ± 0.03
	1.0	0.75 ± 0.03	0.88 ± 0.03
	5.0	0.75 ± 0.02	0.87 ± 0.03
	25.0	0.77 ± 0.03	0.87 ± 0.03

PCA was administered i.p. 30 minutes before the training trial. HBA was administered p.o. 1 hr before the training trial. The results are expressed as mean ± S.E. of 8 rats per group.

HBA might be further studied.

Recent studies support an inhibitory role of the noradrenergic system in learning and memory. Administration of epinephrine and norepinephrine were found to suppress avoidance behavior.[30] There is also a reduction in brain norepinephrine levels after avoidance learning.[31] PCA has been found to induce 5-HT and norepinephrine release in some brain areas.[13] In addition, the mechanism of pindolol action could involve 5-HT_{1A} and β-adrenergic receptors.[19] To distinguish the role of adrenergic receptors on the inhibitory effect of HBA on PCA-induced impairment of the passive avoidance response, we employed the α_1-blocker prazosin, α_2-blocker yohimbine and β-blocker propranolol. Our data revealed that prazosin potentiated the effect of HBA on PCA-mediated impairment of the passive avoidance response, whereas yohimbine and propranolol did not. Though propranolol acts through both 5-HT_{1A} and β-adrenergic receptors, the dose used in the present study should mainly act through β-adrenergic receptors.[32] Biochemical studies have pointed out that stimulation of α_1-receptors might increase the serotonin concentration and α_1-receptor antagonists could decrease the serotonin concentration in the hippocampus and striatum.[33,34] Furthermore, Huang *et al.* have reported that aqueous extracts of GE could decrease the norepinephrine concentration in the rat brain.[35] Therefore, the counteracting effect of HBA on the PCA-induced impairment of the passive avoidance response might be indirectly related to α_1-receptors. The mechanisms of HBA action on the serotonergic and adrenergic systems must be further studied.

Taking all these observations into consideration, we propose that the beneficial effect of HBA on the PCA-induced impairment of the passive avoidance response may be due to

decreased serotonergic activity by 5-HT_{1A} and 5-HT_2 receptors, and also a decrease in the adrenergic activity by α_1-receptors.

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